American Journal of Medical Genetics 63:123–128 (1996)

A Specific Collagen Type II Gene (COL2A1) Mutation Presenting as Spondyloperipheral Dysplasia

Bernhard Zabel, Katja Hilbert, Hartmut Stöß, Andrea Superti-Furga, Jürgen Spranger, and Andreas Winterpacht

Children's Hospital, University of Mainz, D-55101 Mainz, Germany (B.Z., K.H., J.S., A.W.); Institute of Pathology St. Johannisstift, D-33102 Paderborn, Germany (H.S); and Department of Pediatrics, University of Zürich, CH-8032 Zürich, Switzerland (A.S.-F.)

We report on a patient with a skeletal dysplasia characterized by short stature, spondylo-epiphyseal involvement, and brachydactyly E-like changes. This condition has been described as spondyloperipheral dysplasia and the few published cases suggest autosomal dominant inheritance with considerable clinical variability. We found our sporadic case to be due to a collagen type II defect resulting from a specific COL2A1 mutation. This mutation is the first to be located at the C-terminal outside the helical domain of COL2A1. A frameshift as consequence of a 5 bp duplication in exon 51 leads to a stop codon. The resulting truncated C-propeptide region seems to affect helix formation and produces changes of chondrocyte morphology, collagen type II fibril structure and cartilage matrix composition. Our case with its distinct phenotype adds another chondrodysplasia to the clinical spectrum of type II collagenopathies.

KEY WORDS: skeletal dysplasia, COL2A1 defect, spondyloepiphyseal-spondyloperipheral dysplasia, brachydactyly E, C-propedtide, chondrocalcin

INTRODUCTION

The first mutation of the type II collagen gene (COL2A1) was detected in a familial case of spondyloepiphyseal dysplasia congenita SEDC [Lee et al., 1989]. Since then about 30 COL2A1 defects have been described in a spectrum of chondrodysplasias belonging to the SEDC family and the Kniest/Stickler family of skeletal dysplasias [Spranger, 1988]. The type II collagenopathies range from lethal forms (Achondrogenesis II/Hypochondrogenesis) to severe conditions (SEDC, Kniest dysplasia) and mild phenotypes (Stickler dysplasia, mild dominant spondylarthropathy) [Spranger et al., 1994]. For all these disorders heterozygous mutations that map to different parts of the triple helical domain of the COL2A1 gene have been found.

We now describe the first mutation located outside this helical domain in the region of the C-propeptide. Duplication of 5 nucleotides led to a frameshift producing a stop codon at the end of exon 51. A phenotype called spondyloperipheral dysplasia [Kelly et al., 1977] resulted that has previously not been linked to COL2A1. Our observation contributes to the study of the COL2A1 genotype-phenotype correlation and identifies a specific type II defect with distal limb involvement.

CLINICAL REPORT

This girl was born to healthy, nonconsanguineous parents after 40 weeks of gestation with a weight of 3,500 g and a length of 45 cm. A skeletal dysplasia was suspected because of rhizomelic shortening of arms and legs. X-ray findings at 7 months suggested SEDC. Reexamination at the age of 14 years showed short stature (height: 127.4 cm, -5.4 SD), with short and broad fingers, and short toes II-V. Additional findings were a slightly hypoplastic midface with depressed nasal bridge, severe myopia (7 dptr), short neck and trunk, accentuated lumbar lordosis, and limited extension of the elbow joints (Fig. 1). The palate was intact and hearing was normal.

RADIOGRAPHIC FINDINGS

The radiographs showed a spondyloepiphyseal dysplasia with marked involvement of the hands and feet (Fig. 2).

MOLECULAR STUDIES

DNA was isolated from peripheral blood leukocytes. PCR amplification of the COL2A1 exons was carried out with AmpliTaq (Perkin Elmer Cetus) according to the instructions of the manufacturer. Amplification was

Received for publication January 12, 1996.

Address reprint requests to Dr. Bernhard Zabel, Children's Hospital, University of Mainz, Langenbeckstr. 1, D-55101 Mainz, Germany.

Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

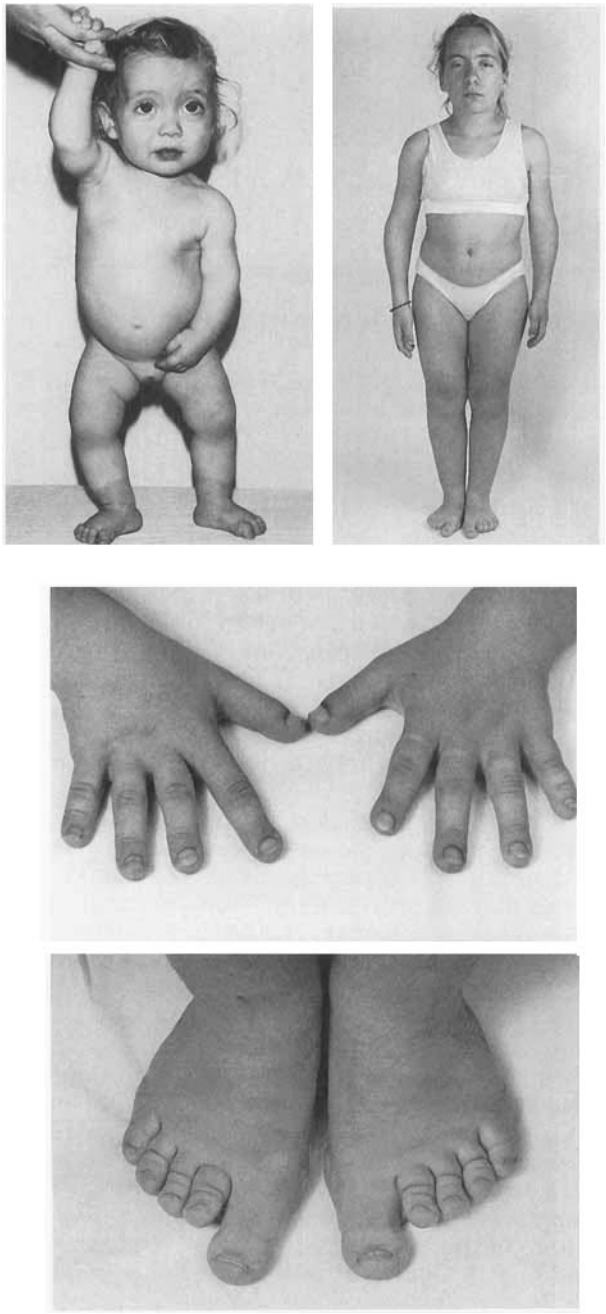

Fig. 1. **Top:** Patient at the age of 18 months (left) and 14 years (right). Brachydactyly **(middle)** and short toes II-V **(bottom)** at the age of 14 years.

performed after an initial heating to 95°C for 30 s, for 10 s at 95°C, 10 s at 60°C, and 10 s at 72°C over 35 cycles in a PTC-100 (MJ Research, Inc.) thermocycler. Mutation detection was performed by non-radioactive multiplex-SSCP analysis exactly as described previously [Winterpacht et al., 1995]. In brief, up to ten PCR products (10 ng each) were pooled, denatured, and separated on a polyacrylamid gel as described by Orita et al. [1989]. After electrophoresis, the gel was blotted onto a Nylon membrane (Amersham Hybond+) and hybridized to the different PCR products, successively. For hybridization, PCR products were used that had been generated by adding alkali labile Dig-11-dUTP (Boehringer Mannheim) to the PCR reaction (0.82 mM dTTP to 0.43 mM Dig-11-dUTP). Prehybridization, hybridization, and stripping procedure was performed as described earlier [Winterpacht et al., 1995]. PCR-products were cloned into plasmid vector pBluescript. Plasmid sequencing was carried out using an ABI 373A automatic sequencing apparatus, with the dye deoxy terminator cycle sequencing kit and the protocols of ABI.

With a set of 43 different primer pairs we amplified all 54 exons of the patient's COL2A1 gene. SSCP analysis demonstrated an additional band at exon 51, in the C-propeptide of collagen II. Sequencing analysis showed a 5 bp duplication of nucleotides 672-676 of the C-propeptide (Fig. 3). The duplication changed the reading frame leading to a premature STOP at the end of exon 51 (codon 1236). The duplication was exclusively present in the affected individual and absent in the healthy parents and in 50 healthy control individuals (data not shown). This strongly indicates that the duplication and the resulting premature translation stop can be seen as the molecular basis of the clinical phenotype. In addition, a G to A point mutation causing a Gly(1205) to Ser conversion was detected. This Gly conversion represents a polymorphism as it was also present in the patient's healthy father and in one of 50 unrelated healthy control individuals.

BIOCHEMICAL ANALYSIS

Preparation of iliac crest biopsy material and analysis of cartilage extracts by SDS-PAGE and silver staining disclosed a marked reduction of collagen type II to about 25% of its normal content. Collagen mobility was unaltered, thus showing no signs of post-translational overmodification (data not shown).

MORPHOLOGICAL STUDIES

Iliac crest biopsy samples were fixed in phosphate-buffered 4% paraformaldehyde solution. For histological and immunhistochemical investigation part of the biopsy material was decalcified by 5% formic acid, embedded in paraffin [Winterpacht et al., in press]. For electron microscopy the tissue was embedded undecalcified in low-viscosity epoxy resin using a modification of Schulz [1977]. Ultrathin sections were contrasted by uranyl acetate and lead citrate.

Histological examination of cartilage biopsy material showed areas containing few and irregularly distributed chondrocytes of different size and with cytoplasmatic inclusions. Electron microscopy showed (Fig.4) chondrocytes with markedly dilated rough endoplasmatic reticulum filled with fine granular material and an enlarged Golgi region. The cartilage matrix was composed of a collagen fiber net of varying density and with significant variability in fiber diameter. The observed morphologic changes were compatible with a defect of cartilage tissue with structural alterations of chondrocytes and cartilage matrix composition.

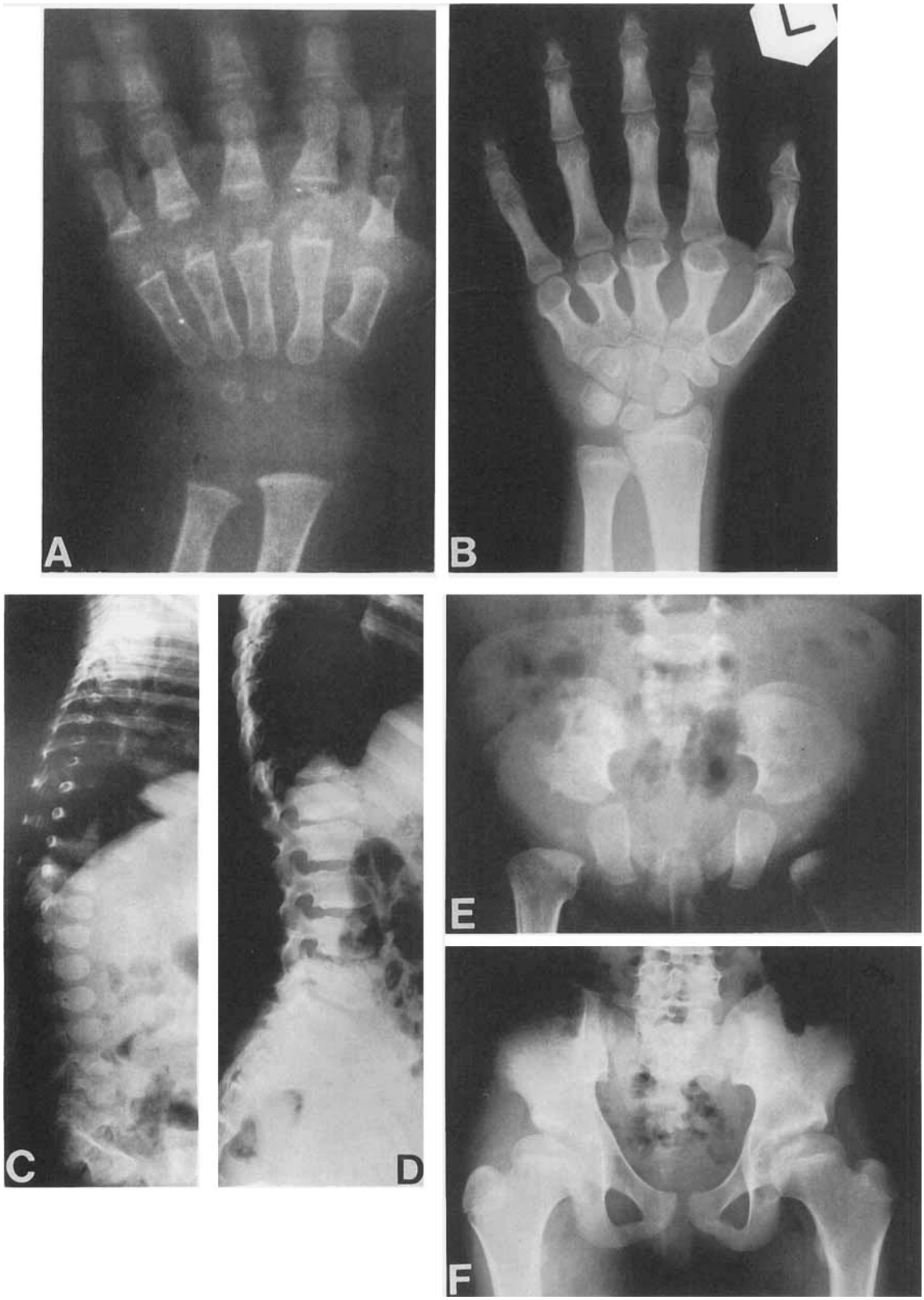

Fig. 2. **A:** (7 months) The carpal bones are small but epiphyseal ossification of the tubular bones is markedly advanced. **B:** (14 years) The metacarpals II–V are short with globular ends and flattened distal articular surfaces. All distal phalanges are short. Ossification is advanced by approximately 1 year. **C:** (7 months) Note ovoid (immature) appearance of the vertebral bodies. **D:** (14 years) The vertebral bodies are flat with irregular upper and lower plates. **E:** (7 months) The ilia are short in their craniocaudal dimension. The acetabula are horizontal with medial and lateral spurs. **F:** (14 years) The capital femoral epiphyses are flat and the necks in valgus position.

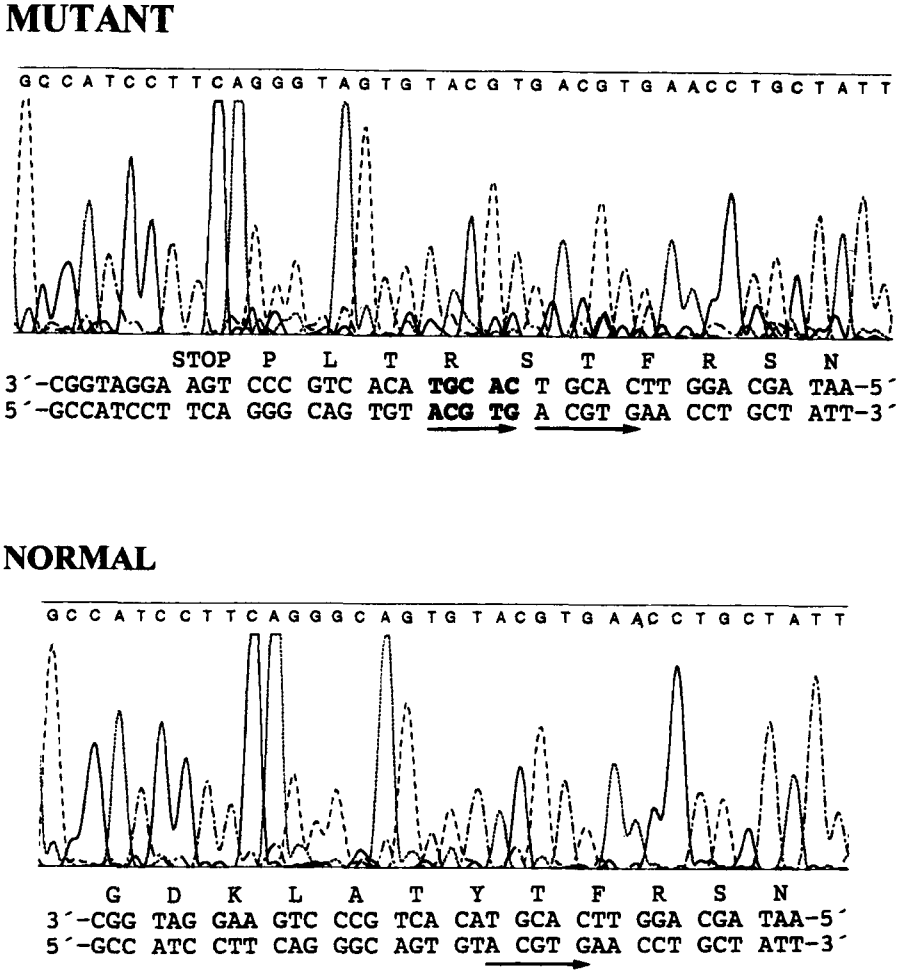

Fig. 3. Sequence analysis of the COL2A1 gene (part of mutant and normal exon 51). **Top:** Duplication of 5 bp (nucleotides 672-676 of the C-propeptide, arrows). **Bottom:** Normal sequence.

DISCUSSION

The term 'spondyloperipheral dysplasia' was first used by Kelly et al. [1977] for a disorder with generalized platyspondyly, premature osteoarthrosis of the proximal femora, short hands and feet. There was marked intrafamilial variability of the hand changes with abnormally short tubular hand bones in the father but brachymetacarpy V only and normal distal phalanges in the daughter. In spite of some differences, this intrafamilial variability suggests that the families reported by Vanek [1983], Sybert et al. [1979], Sorge et al. [1995], and our patient have the same condition. A patient described by Ioan et al. [1993] as spondyloperipheral dysplasia clearly had a different disease.

The molecular defect in our patient (a frameshift with subsequent stop codon as result of a 5 bp duplication in exon 51 of the COL2A1 gene) is most likely to have caused the phenotype as it was not found in the patient's parents and in a series of unaffected control persons.

Biochemical analysis of the patient's cartilage showed a significantly low amount of type II collagen. Morphological examination of the cartilage documented large inclusions with markedly dilated rough endoplasmatic reticulum containing type II collagen material. Moreover, disorganized cartilage with sparsely distributed type II collagen and fibrils highly variable in diameter could be detected. Taken together, biochemical and histological data suggest a defect affecting the processing of collagen type II with intracellular retention and the secretion of some mutant protein leading to disorganized cartilage with matrix deposition of structurally altered collagen fibrils.

The mutation in the COL2A1 gene region that encodes the carboxyl-terminal domain results in a premature translation stop. As consequence, the α1(II)-chain is truncated in the C-propeptide region. The deleted part of the C-propeptide contains two of the four Cys residues which play a crucial role inducing the helix assembly and building disulfide bonds between the three procollagen chains to form the helical molecule [Kielty et al., 1993]. To explain the phenotypic consequences, one might hypothesize that the mutation leads to the synthesis of chains of altered structure which prevents

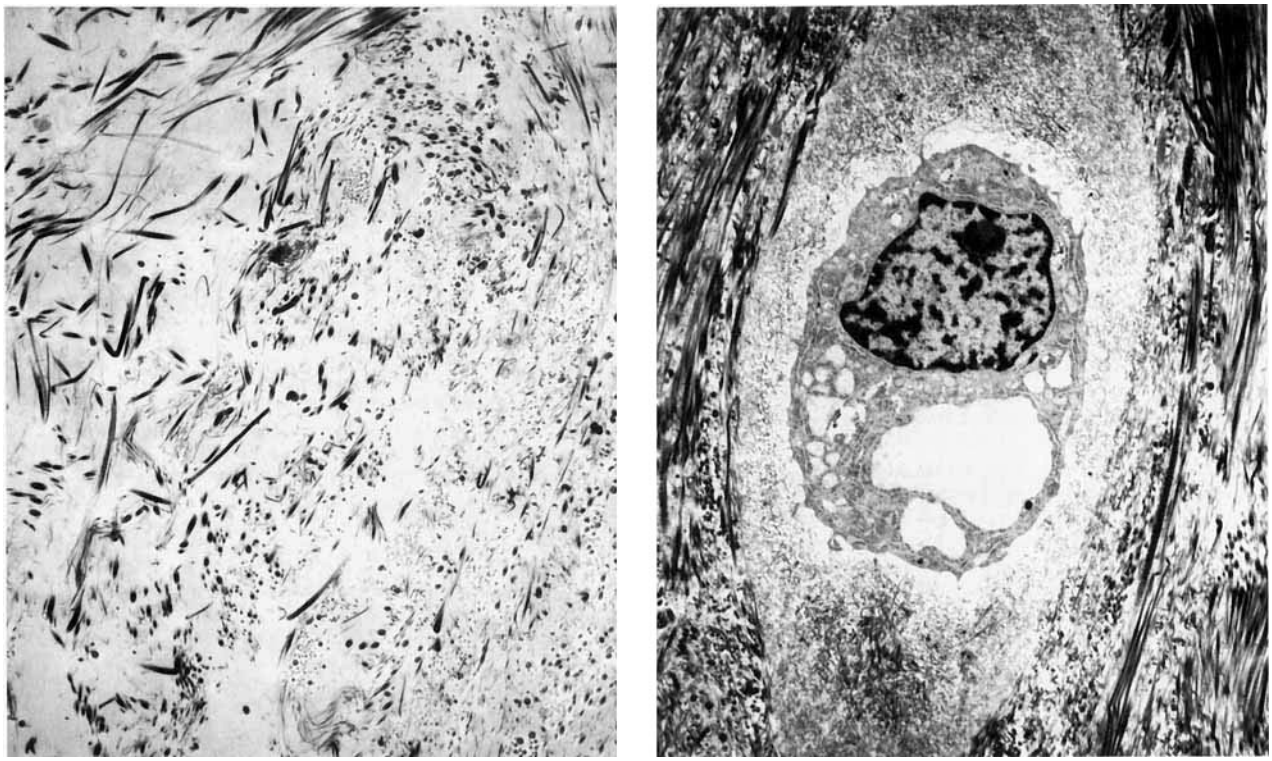

Fig. 4. Electron microscopy of iliac crest biopsy. **Left:** Irregular extracellular matrix with collagen fiber net of varying density and with significant variability in fiber diameter (EM X3,000). **Right:** Chondrocyte with large dilated cisternae of rough endoplasmatic reticulum (EM X3,000).

their association into type II collagen trimers. Exclusion of the abnormal chains from the type II collagen trimers would result in a 50% reduction of type II collagen in the matrix and the phenotype could be explained in terms of haploinsufficiency. An alternative would be a dominant gain of function mutation mechanism. In this model the abnormal type II collagen chains would either associate with the normal chains and produce type II collagen trimers of altered structure that have deleterious intracellular or extracellular effects, or the abnormal chains would not be able to associate into trimers but, their presence within the cell might have a negative effect on its function. Similar ideas were developed for collagen type X, a homotrimer whose structure might be partly comparable to type II collagen, although there are obvious differences concerning function and supramolecular organisation. The models are proposed to link the exclusively carboxyl-terminal defects of the type X collagen gene (COL10A1) to the resulting phenotype of the metaphyseal chondrodysplasia type Schmid [Wallis et al. in press].

A collagen type II defect resulting in a reduced amount of cartilage collagen should present as mild, Stickler dysplasia-like phenotype, or, if the defective α1(II)-chains are included in the trimer formation, in a more severe SEDC-like disorder [Spranger et al., 1994]. The peculiar spondyloperipheral dysplasia in our patient may be related to a specific function of the C-propeptide. A mutation truncating the C-propeptide may impair a specific role of this peptide, which is also known as chondrocalcin [Poole et al., 1984; Alini et al., 1992]. Chondrocalcin accumulates in the hypertrophic zones of the growth plate and seems to promote mineralisation in this zone. A defective C-propeptide may cause premature closure of specific growth plates resulting in abnormally short tubular bones. The cone shaped epiphyses seen in some patients [Kelly et al., 1977] support this hypothesis.

Our case, together with other observations of spondyloperipheral dysplasia should be helpful to further study the COL2A1 genotype-phenotype correlation, especially to identify specific type II defects with distal limb involvement. Using antibodies to the C-propeptide of type II collagen [Poole et al., 1984] it should be possible to clarify the speculations about the function of chondrocalcin.

ACKNOWLEDGMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to B.Z. and A.W. and by a Concerted Action grant from the Commission of the European Communities for the study of the chondrodysplasias.

REFERENCES

Alini M, Matsui Y, Dodge GR, Poole AR (1992): The extracellular matrix of cartilage in the growth plate before and during calcification:

Changes in composition and degradation of type II collagen. Calcif Tissue Int 50:327–335.

Ioan DM, Popa M, Fryns JP (1993): An unclassifiable type of spondyloperipheral epiphyseal dysplasia associated with 21 trisomy. Genet Counseling 4:59–62.

Kelly TE, Lichtenstein JR, Dorst JP (1977): An unusual familial spondyloepiphyseal dysplasia: 'Spondyloperipheral dysplasia.' New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS XIII(3):149–165.

Kielty CM, Hopkinson I, Grant ME (1993): The collagen family: Structure, assembly, and organization in the extra-cellular matrix. In: Royce PM, Steinmann B (eds): "Connective Tissue and its Heritable Disorders." New York: Wiley Liss, pp 103–148.

Lee B, Vissing H, Ramirez F, Rogers D, Rimoin D (1989): Identification of the molecular defect in a family with spondyloepiphyseal dysplasia. Science 244:978–980.

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989): Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879.

Poole AR, Pidoux I, Reiner A, Choi H, Rosenberg LC (1984): Association of an extracellular protein (chondrocalcin) with the calcification of cartilage in enchondral bone formation. J Cell Biol 98: 54–65.

Schulz A (1977): A reliable method of preparing undecalcified human bone biopsies for electron microscopic investigation. Microscopia Acta 7–18.

Sorge G, Ruggieri M, Lachman RS (1995): Spondyloperipheral dysplasia. Am J Med Genet 59:139–142.

Spranger J (1988): Bone dysplasia 'families'. Pathol Immunopathol Res 7:76–80.

Spranger J, Winterpacht A, Zabel B (1994): The type II collagenopathies: A spectrum of chondrodysplasias. Eur J Pediatr 153:56–65.

Sybert VP, Byers PH, Hall JG (1979): Variable expression in a dominantly inherited skeletal dysplasia with similarities to brachydactyly E and spondyloepiphyseal-spondyloperipheral dysplasia. Clin Genet 15:160–166.

Vanek J: (1983): Spondyloperipheral dysplasia. J Med Genet 20: 117–121.

Wallis GA, Rash B, Sykes B, Bonaventure J, Maroteaux P, Zabel B, Wynne-Davies R, Grant ME, Boot-Handford RP (1996): Mutations within the gene encoding the α1(X) chain of type X collagen (COL10A1) cause metaphyseal chondrodysplasia type Schmid but not several related forms of chondrodysplasia. J Med Genet (in press).

Winterpacht A, Hilbert K, Schwarze U, Zabel B (1995): Nonradioactive multiplex-SSCP analysis: Detection of a new type II procollagen gene (COL2A1) mutation. Hum Genet 95:437–439.

Winterpacht A, Superti-Furga A, Schwarze U, Stöß H, Steinmann B, Spranger J, Zabel B (1996): The deletion of six amino acids at the C-terminus of the α1(II) chain causes overmodification of type II and type XI collagen: further evidence for the association between small deletions in COL2A1 and Kniest dysplasia. J Med Genet (in press).